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### **Homocysteine and tissue factor pathway inhibitor levels in patients with Fabry's disease**

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- 6 Mariani MA, Gu YJ, Boonstra PW, Grandjean JG, van Oeveren W, Ebels T. Procoagulant activity after off-pump coronary operation: is the current anticoagulation adequate? *Ann Thorac Surg* 1999; **67**: 1370–5.
- 7 Murkin JM, Newman SP, Stump DA, Blumenthal JA. Statement of consensus on assessment of neurobehavioral outcomes after cardiac surgery. *Ann Thorac Surg* 1995; **59**: 1289–95.
- 8 Stump DA. Selection and clinical significance of neuropsychologic tests. *Ann Thorac Surg* 1995; **59**: 1340–4.
- 9 Kim KB, Lim C, Lee C, Chae IH, Oh BH, Lee MM, Park YB. Off-pump coronary artery bypass may decrease the patency of saphenous vein grafts. *Ann Thorac Surg* 2001; **72**: S1033–7.
- 10 Nierich AP, Diephuis J, Jansen EW, Borst C, Knappe JT. Heart displacement during off-pump CABG: how well is it tolerated? *Ann Thorac Surg* 2000; **70**: 466–472.
- 11 Kapetanakis EI, Stamou SC, Dullum MK, Hill PC, Haile E, Boyce SW, Bafi AS, Petro KR, Corso PJ. The impact of aortic manipulation on neurologic outcomes after coronary artery bypass surgery: a risk-adjusted study. *Ann Thorac Surg* 2004; **78**: 1564–71.
- 12 van Dijk D, Moons KG, Keizer AM, Jansen EW, Hijman R, Diephuis JC, Borst C, de Jaegere PP, Grobbee DE, Kalkman CJ. Association between early and three month cognitive outcome after off-pump and on-pump coronary bypass surgery. *Heart* 2004; **90**: 431–4.

## Homocysteine and tissue factor pathway inhibitor levels in patients with Fabry's disease

S. FEDI,\* F. GENSINI,† A. M. GORI,\* R. ABBATE\* and W. BORSINI‡

\*Department of Medical and Surgical Critical Care, Thrombosis Center, Azienda Ospedaliero-Universitaria Careggi, University of Florence, Florence, Italy; †Department of Clinical Pathology, Genetic Unit, University of Florence, Italy; and ‡Department of Neurological Sciences, University of Florence, Italy

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Cerebrovascular disorders are a frequent clinical manifestation of Fabry's disease, a rare inborn X-linked (locus Xq22) recessive deficiency of the lysosomal enzyme alpha-galactosidase A. The enzymatic defect results in progressive accumulation of neutral glycosphingolipids in the lysosomes of vascular endothelial and other cells throughout the body [1].

The incidence of classical disease has been estimated to be about 1:40000 and clinical features include involvement of heart, kidney, skin, nervous system, and the eye. Cerebrovascular manifestations consist of large-vessel ectasia, large-vessel occlusive disease, and small-vessel occlusive disease [2].

The occurrence of clinical manifestations shows phenotypic variance not only associated to different genetic mutations, but also within different members of affected families [2]. In the general population, increased susceptibility to cerebrovascular ischemic events has been described in relation to prothrombotic risk factors [3].

Aim of this study was to evaluate the possible role of prothrombotic risk factors in the clinical presentation of Fabry's disease.

Correspondence: Sandra Fedi, Department of Medical and Surgical Critical Care Medicine, Section of Clinical Medicine and Cardiology, Thrombosis Centre, University of Florence, Viale Morgagni, 85, Florence, Italy.

Tel.: +39 055 4279420; fax: +39 055 4279418; e-mail: s.fedi@dac.unifi.it

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We investigated three unrelated Italian families for a total of nine Fabry patients (four hemizygotes and five heterozygotes, age ranges 21–55 years) compared with 27 Italian control subjects (12 males and 15 females; age ranges 21–55 years). Four of nine Fabry patients (two hemizygotes and two heterozygotes) had recurrent strokes and one of them suffers also from Parkinson symptoms. Magnetic resonance imaging was abnormal in all the patients and showed lacunar infarctions in the periventricular white matter, basal ganglia and pons [4]. The four patients with cerebrovascular complications did not present cardiovascular risk factors (e.g. smoking, dyslipidemia, diabetes, hypertension, cardiopathy, coagulopathies and vasculitis).

Citrated plasma for protein C (PC) activity (DADE-Behring, Dudingen, France), protein S (PS) activity (Instrumentation Laboratory, Milan, Italy), lipoprotein(a) [Lp(a)], tissue factor (TF) and tissue factor pathway inhibitor (TFPI) (TintElize Lp(a), Biopool, Umea, Sweden; IMUBIND Tissue Factor ELISA Kit; IMUBIND Total TFPI ELISA Kit, American Diagnostica Inc, Greenwich, CT, USA) were collected and stored at –80 °C. Anticardiolipin antibodies (aCL) IgG/IgM and anti-Beta2-Glycoprotein I (anti-Beta2-GPI) IgG/IgM (aCL: First Cardiolipin, Eurospital, Trieste, Italy; anti-Beta2-GPI: Arnika, Milan, Italy) were detected in serum samples. Homocysteine (Hcy) levels were detected in EDTA samples by the FPIA method (IMX Abbot Laboratories, Abbott Park, IL, USA). Factor V G1691A, Factor II G20210A and MTHFR C677T polymorphisms were identified as previously described [5–7].

Hemizygote (■) and heterozygote (●) Fabry patients with cerebrovascular manifestations  
 Hemizygote (▨) and heterozygote (◐) Fabry patients with cerebrovascular manifestations  
 Male (□) and female (○) control subjects

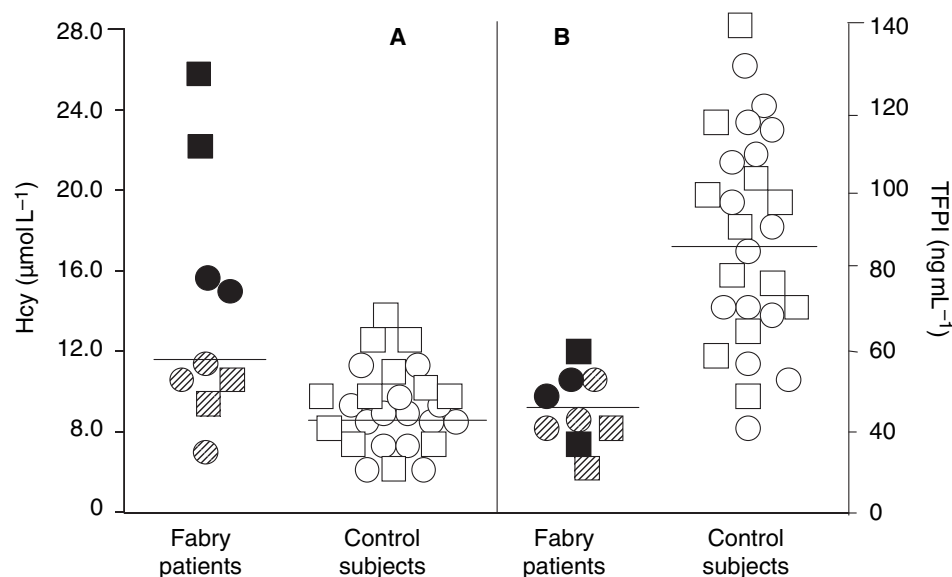


Fig. 1. Homocysteine plasma levels (1A) and TFPI plasma levels (1B) in Fabry's disease patients.

The non-parametric Mann–Whitney *U*-test for unpaired data was used for comparisons between single groups. For genetic analysis, Hardy–Weinberg equilibrium was assessed using  $\chi^2$  analysis.

No patient showed alterations of blood coagulation inhibitors, Lp(a) and the presence of antiphospholipid antibodies and prothrombotic mutations (data not shown). Homocysteine median value was higher in patients than in controls [11 (6.5–25.6)  $\mu\text{mol L}^{-1}$  vs. 8.8 (4.8–13.8)  $\mu\text{mol L}^{-1}$ ,  $P = 0.005$ ] (Fig. 1A). Hyperhcy (above the 95th percentile cut-off of the control population;  $M = 15.5 \mu\text{mol L}^{-1}$ ,  $F = 12.5 \mu\text{mol L}^{-1}$ ) was found in the 4/4 patients with cerebrovascular disease. Median folic acid and vitamin B6 values were lower in patients than in controls [7.9 (3.2–18.8)  $\text{nmol L}^{-1}$  and 22.3 (11.7–50.5)  $\text{nmol L}^{-1}$  vs. 13.8 (7.0–24.5)  $\text{nmol L}^{-1}$  and 40.1 (15.8–68.4)  $\text{nmol L}^{-1}$  respectively  $P < 0.05$ ], whereas no difference was found in vitamin B12 levels. Tissue factor and TFPI levels were higher and respectively lower in Fabry patients than controls [TF: 152 (46–274)  $\text{pg mL}^{-1}$  vs. 107 (22–313)  $\text{pg mL}^{-1}$ ;  $P = 0.18$ ; TFPI: 43.0 (35.0–60.0)  $\text{ng mL}^{-1}$  vs. 86.3 (38.2–151.0)  $\text{ng mL}^{-1}$ ,  $P < 0.0001$  (Fig. 1B)].

These results suggest that hyperhcy may contribute to the cerebral ischemic events in Fabry patients. Hyperhcy may contribute to the endothelial dysfunction, which has been demonstrated in Fabry's disease [8,9]. In cerebral blood vessels of patients with Fabry's disease, a chronic alteration of the nitric oxide (NO) pathway has been demonstrated [8,10] and this condition may be influenced by Hcy, which increases the oxidative degradation of NO [11]. Low folate and vitamin B6 levels may be low due to inadequate dietary intake, malabsorption, increased utilization or to effects of drugs [12–14]. In

this study, gastrointestinal manifestations were reported in three of four patients who suffered from cerebrovascular events, but malabsorption may take place in the absence of clinical signs.

In Fabry's disease, extensive clinical heterogeneity has been related to different genetic mutations, of which more than 110 variants have been described [1,15]. Interestingly, we observed that, in one of the families investigated, the three out of six patients affected by Fabry's disease, who suffered from cerebrovascular ischemic events, were found to be hyperhcy, suggesting the possible role of hyperhcy as mechanism involved in increasing the risk of occurrence of cerebrovascular events.

In this study, TFPI levels have been found to be low in all the patients so suggesting a diffuse endothelial alterations associated to the disease *per se*. Tissue factor pathway inhibitor plays a primary role in regulating TF-induced coagulation and significantly contributes to the anticoagulant properties of the endothelium [16]. Tissue factor pathway inhibitor decrease has been reported in conditions in which blood clotting activation occurs [17]. Low levels of plasma TFPI in Fabry's disease may be attributed to the endothelial dysfunction and/or consumption of the endothelial cell-associated TFPI owing to the proinflammatory and procoagulant profile of the Fabry patients. TF may have a role in determining the procoagulant profile to which other mechanisms participate. In conclusion, the present study shows that Hcy may have a role in the occurrence of cerebrovascular events in Fabry patients and that reduced vitamin availability is involved. The possible utility of checking for Hcy and vitamin levels, in particular in the presence of gastrointestinal

events, and the opportunity of vitamin administration deserves confirmation in a larger group of patients with Fabry's disease.

## References

- Desnick RJ, Ioannou YA, Eng CM. Alpha-galactosidase A deficiency: Fabry disease. In: Beaudet A, Sly W, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*, 8th edn. New York: Mc Graw-Hill, 2001: 3733–74.
- Peters FPJ, Vermeulen A, Kho TL. Anderson-Fabry's disease:  $\alpha$ -galactosidase deficiency. *Lancet* 2001; **357**: 138–40.
- The Homocysteine Studies Collaboration. Homocysteine and risk of ischemic disease and stroke: a meta-analysis. *JAMA* 2002; **288**: 2015–22.
- Borsini W, Giuliaci G, Torricelli F, Pelo E, Martinelli F, Scordo MR. Anderson-Fabry disease with cerebrovascular complications in two Italian families. *Neurol Sci* 2002; **23**: 49–53.
- Bertina RM, Koeleman BP, Koster T, Rosendaal F, Dirven RJ, De Ronde H. Mutation in blood coagulation factor V associated with resistance to activate protein C. *Nature* 1994; **369**: 64–7.
- Poort SW, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996; **88**: 3698–703.
- Abbate R, Sardi I, Pepe G, Marcucci R, Brunelli T, Prisco D, Fatini C, Capanni M, Simonetti I, Gensini GF. The high prevalence of thermolabile 5–10 methylenetetrahydrofolate reductase (MTHFR) in Italians is not associated to an increased risk for coronary artery disease (CAD). *Thromb Haemost* 1998; **79**: 727–30.
- Altarescu G, Moore DF, Pursely R, Campia U, Goldstein S, Bryant M, Panza JA, Schiffmann R. Enhanced endothelium-dependent vasodilatation in Fabry disease. *Stroke* 2001; **32**: 1559–62.
- DeGraba T, Azhar S, Dignat-George F, Brown E, Boutière B, Altarescu G, McCarron R, Schiffmann R. Profile of endothelial and leukocyte activation in Fabry patients. *Ann Neurol* 2000; **47**: 229–33.
- Moore DF, Scott LTC, Gladwin MT, Altarescu G, Kaneski C, Suzuki K, Pease-Fye M, Ferri R, Brady RO, Herscovitch P, Schiffmann R. Regional cerebral hyperfusion and nitric oxide pathway dysregulation in Fabry disease. Reversal by enzyme replacement therapy. *Circulation* 2001; **104**: 1506–12.
- Upchurch GR, Welch GN, Fabian AJ, Pigazzi A, Keaney JF, Loscalzo J. Stimulation of endothelial nitric oxide production by homocyst(e)ine. *Atherosclerosis* 1997; **132**: 177–85.
- Demuth K, Germain DP. Endothelial markers and homocysteine in patients with classic Fabry disease. *Acta Paediatr Suppl* 2002; **91**: 57–61.
- Selhub J. Homocysteine metabolism. *Ann Rev Nutr* 1999; **19**: 217–57.
- Desouza C, Kleeber M, McNamara DB, Fonseca V. Drugs affecting homocysteine metabolism. *Drugs* 2002; **62**: 605–16.
- Froissart R, Guffon N, Vanier MT, Desnick RJ, Maire I. Fabry disease: D313Y is an alpha-galactosidase A sequence variant that causes pseudodeficient activity in plasma. *Mol Genet Metab* 2003; **80**: 307–14.
- Bajaj MS, Birktoft JT, Steer SA, Bajaj SP. Structure and biology of tissue factor pathway inhibitor. *Thromb Haemost* 2001; **86**: 959–72.
- Blann AD, Amiral J, McCollum CN, Lip CYH. Differences in free and total tissue factor pathway inhibitor, and tissue factor in peripheral artery disease compared to healthy controls. *Atherosclerosis* 2000; **152**: 29–34.

# Further evidence that fibrillar collagen is unable to promote platelet shape change and aggregation in the absence of secondary agonists

P. OHLMANN, A. ECKLY, P. MANGIN, F. LANZA and C. GACHET  
INSERM U311, EFS-Alsace, Strasbourg, France

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Platelet activation by collagen involves multiple receptors including glycoprotein (GP)VI and the integrin  $\alpha_2\beta_1$  as primary receptors, secondary agonists such as released ADP acting on both P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors and thromboxane A<sub>2</sub> (TXA<sub>2</sub>) activating Tp receptor. Intracellularly, PLC $\gamma$ 2

Correspondence: Christian Gachet, INSERM U311, Etablissement Français du Sang-Alsace, 10, rue Spielmann, B. P. No. 36, 67065 Strasbourg Cedex, France.

Tel.: +33 3 88 21 25 25; fax: +33 3 88 21 25 21; e-mail: christian.gachet@efs-alsace.fr

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downstream of GPVI, as well as the Gq and G<sub>13</sub>  $\alpha$ -subunits of the heterotrimeric G-proteins have been demonstrated to be key signaling elements during platelet activation by collagen. We recently have shown that simultaneous inhibition of TXA<sub>2</sub> formation by aspirin and blockade of the P2Y<sub>1</sub> receptor by gene targeting or using a selective P2Y<sub>1</sub> antagonist resulted in complete loss of shape change in platelets exposed to a high concentration (100  $\mu$ g mL<sup>-1</sup>) of fibrillar collagen [1]. Scanning electron microscopic (SEM) analysis provided clear images of platelets in direct contact with collagen fibers that had not undergone shape change and showed a typical resting discoid ultrastructure. These data led us to propose that the P2Y<sub>1</sub> receptor plays a key role in collagen-induced platelet